

## **IN THE CLAIMS:**

The following listing will replace all prior versions and listings of the claims.

1-38. (Cancelled)

39. (Currently Amended) A method of producing human neural progenitor cells from human embryonic stem (hES) cells *in vitro*, said method comprising:

culturing undifferentiated pluripotent hES cells for 2-3 weeks ~~under adherent conditions on fibroblast feeder cells~~, so as to generate differentiating cells; and

culturing said differentiating cells in the presence of serum free medium supplemented with growth factors which include epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF), thereby obtaining neural progenitor cells, wherein said neural progenitor cells are capable of further differentiation into neurons, into oligodendrocytes, and into astrocytes, and wherein said neural progenitor cells express polysialated N-CAM, nestin, vimentin and the transcription factor Pax-6.

40-43. (Cancelled)

44. (Previously Presented) The method according to claim 39 wherein said undifferentiated pluripotent hES cells are prepared according to a method comprising:

obtaining an *in vitro* fertilised human embryo and growing the embryo to a blastocyst stage of development;

removing inner cells mass (ICM) cells from the embryo;

culturing ICM cells under conditions which do not induce extraembryonic differentiation and cell death, and promote proliferation of undifferentiated stem cells; and

recovering stem cells.

45. (Previously Presented) The method according to claim 44 wherein the method for preparing said undifferentiated pluripotent hES cells is further characterized by:

culturing the ICM cells on a fibroblast feeder layer to promote proliferation of embryonic stem cells prior to recovering the stem cells from the feeder layer, wherein the fibroblast feeder cells are arrested in their growth.

replating the stem cells from the fibroblast feeder layer onto another fibroblast feeder layer; and

culturing the stem cells for a period sufficient to promote proliferation of morphologically undifferentiated stem cells.

46-50. (Cancelled)

51. (Currently amended) A method of inducing differentiation of neural progenitors into neurons, said method comprising:

~~obtaining a source of neural progenitor cells derived from human pluripotent embryonic stem cells *in vitro*~~; culturing undifferentiated pluripotent hES cells for 2-3 weeks on fibroblast feeder cells, so as to generate differentiating cells;

culturing said differentiating cells in the presence of serum free medium supplemented with growth factors which include epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF), thereby obtaining neural progenitor cells, wherein said neural progenitor cells are capable of further differentiation into neurons, into oligodendrocytes, and into astrocytes, and wherein said neural progenitor cells express polysialated N-CAM, nestin, vimentin and the transcription factor Pax-6;

culturing the neural progenitor cells on an adhesive substrate in the presence of a serum free media and growth factors; and

inducing the neural progenitor cells to differentiate into neurons by withdrawal of the growth factors; and determining an expression of a neuronal cell marker.

52-55. (Cancelled)

56. (Currently Amended) A method of inducing differentiation of neural progenitors into neurons said method comprising:

~~obtaining a source of neural progenitor cells, wherein said neural progenitor cells are derived from human pluripotent embryonic stem cells *in vitro*~~; culturing undifferentiated pluripotent hES cells for 2-3 weeks on fibroblast feeder cells, so as to generate differentiating cells;

culturing said differentiating cells in the presence of serum free medium supplemented

with growth factors which include epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF), thereby obtaining neural progenitor cells, wherein said neural progenitor cells are capable of further differentiation into neurons, into oligodendrocytes, and into astrocytes, and wherein said neural progenitor cells express polysialated N-CAM, nestin, vimentin and the transcription factor Pax-6; and

culturing the neural progenitor cells on an adhesive substrate which comprises poly-D-lysine and laminin in the presence of a serum free media, to induce differentiation of the neural progenitor cells into neurons; and determining an expression of a neuronal cell marker.

57. (Previously presented) The method according to claim 56 wherein after culturing the neural progenitor cells on an adhesive substrate in the presence of a serum free media, the cells are further cultured in the presence of retinoic acid.

58-59. (Cancelled)

60. (Currently amended) A method of inducing differentiation of neural progenitors into oligodendrocytes or astrocytes, said method comprising:

~~producing neural progenitor cells according to claim 39~~ culturing undifferentiated pluripotent hES cells for 2-3 weeks on fibroblast feeder cells, so as to generate differentiating cells;

culturing said differentiating cells in the presence of serum free medium supplemented with growth factors which include epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF), thereby obtaining neural progenitor cells, wherein said neural progenitor cells are capable of further differentiation into neurons, into oligodendrocytes, and into astrocytes;

culturing the neural progenitor cells in serum free medium in the presence of PDGF-AA and bFGF; and

plating the neural progenitor cells on an adhesive substrate which comprises poly-D-lysine and fibronectin, in serum free medium without PDGF-AA or bFGF, thereby inducing differentiation of the neural progenitor cells into oligodendrocytes or astrocytes.

61. (Currently amended) A method of inducing differentiation of neural progenitors into oligodendrocytes or astrocytes, said method comprising:

~~producing neural progenitor cells according to claim 39~~culturing undifferentiated pluripotent hES cells for 2-3 weeks on fibroblast feeder cells, so as to generate differentiating cells;

culturing said differentiating cells in the presence of serum free medium supplemented with growth factors which include epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF), thereby obtaining neural progenitor cells, wherein said neural progenitor cells are capable of further differentiation into neurons, into oligodendrocytes, and into astrocytes;

culturing the neural progenitor cells in serum free medium in the presence of PDGF-AA and bFGF;

plating the neural progenitor cells on an adhesive substrate which comprises poly-D-lysine and fibronectin;

culturing the neural progenitor cells in serum free medium in the presence of PDGF-AA, bFGF and T3; and

inducing differentiation of the neural progenitor cells by withdrawing PDGF-AA and bFGF in the medium.

62-66. (Cancelled)

67. (Currently Amended) The method according to claim 39 wherein said culturing said differentiating hES cells eliminates non-neural cells.

68-93. (Cancelled)

94. (Previously Presented) The method of claim 39, wherein the neural progenitor cells are cultured as monolayers or spheres.

95-100. (Cancelled)

101. (Previously Presented) The method of claim 39, wherein said culturing undifferentiated pluripotent hES cells for 2-3 weeks does not induce extra embryonic differentiation or cell death.

102-104. (Cancelled)

105. (Currently Amended) The method of claim 39, further comprising selecting from said differentiating cells, cells destined to give rise to neural progenitor cells ~~based on cell morphology.~~

106. (Cancelled)

107. (Previously Presented) The method of claim 51, wherein said neuronal cell marker is selected from the group consisting of 200 kDa neurofilament protein, 160 kDa neurofilament protein, MAP2a+b, glutamate, synaptophysin, glutamic acid decarboxylase and  $\beta$ -tubulin.

108. (Currently Amended) A method of producing human neural progenitor cells from human embryonic stem (hES) cells *in vitro*, the method comprising:

culturing isolated undifferentiated pluripotent hES cells in serum free medium supplemented with growth factors which include epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF), thereby obtaining neural progenitor cells, wherein said neural progenitor cells are capable of further differentiation into neurons, into oligodendrocytes, and into astrocytes, and wherein said neural progenitor cells express polysialated N-CAM, nestin, vimentin and the transcription factor Pax-6.

109. (Previously Presented) The method of claim 39, wherein said culturing undifferentiated pluripotent hES cells does not generate embryoid bodies.

110. (Previously Presented) A method of producing human neural progenitor cells from human embryonic stem (hES) cells *in vitro*, the method comprising:

culturing undifferentiated pluripotent hES cells so as to generate differentiating hES cells and not generate embryoid bodies; and

culturing said differentiating cells in the presence of serum free medium supplemented with growth factors which include epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF), thereby obtaining neural progenitor cells, wherein said neural progenitor cells are capable of further differentiation into neurons, into oligodendrocytes, and into astrocytes, and wherein said neural progenitor cells express

polysialated N-CAM, nestin, vimentin and the transcription factor Pax-6.

111. (Previously Presented) The method of claim 39, wherein said culturing undifferentiated pluripotent hES cells is continuous.

112. (Previously Presented) The method according to claim 56 wherein said neurons are mature neurons.

113. (Previously Presented) The method of claim 56, wherein said neuronal cell marker is selected from the group consisting of 200 kDa neurofilament protein, 160 kDa neurofilament protein, MAP2a+b, glutamate, synaptophysin, glutamic acid decarboxylase and  $\beta$ -tubulin.

114. (New) A method of inducing differentiation of neural progenitors into neurons, said method comprising:

- culturing undifferentiated pluripotent hES cells so as to generate differentiating hES cells and not generate embryoid bodies;

- culturing said differentiating cells in the presence of serum free medium supplemented with growth factors which include epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF), thereby obtaining neural progenitor cells, wherein said neural progenitor cells are capable of further differentiation into neurons, into oligodendrocytes, and into astrocytes;

- culturing the neural progenitor cells on an adhesive substrate in the presence of a serum free media and growth factors; and

- inducing the neural progenitor cells to differentiate into neurons by withdrawal of the growth factors.

115. (New) A method of inducing differentiation of neural progenitors into neurons said method comprising:

- culturing undifferentiated pluripotent hES cells so as to generate differentiating hES cells and not generate embryoid bodies;

- culturing said differentiating cells in the presence of serum free medium supplemented with growth factors which include epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF), thereby obtaining neural progenitor cells, wherein

said neural progenitor cells are capable of further differentiation into neurons, into oligodendrocytes, and into astrocytes; and

culturing the neural progenitor cells on an adhesive substrate which comprises poly-D-lysine and laminin in the presence of a serum free media, to induce differentiation of the neural progenitor cells into neurons.